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1. Monti, PAACR Anuual Metting 1977, v 38(0), p193
2. Monti, PAACR Annual Meeting 1995, v36, p387
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2304

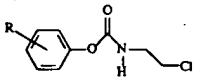
Cytotoxicity of Tempol, a piperidine nitroxide spin label, against different neoplastic and non-neoplastic cell lines. Elena Monti, Marzia Gariboldi, Rosanna Supino*, Francesco Piccinini. Inst of Pharmacology, University of Milan; *Istituto Nazionale Tumori, Milan, Italy.

The stable nitroxide Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) is widely used as probe in biophysical studies and was recently reported to act as radioprotector in mice. The possible cytotoxic effects of Tempol were tested on a panel of human and rodent cell lines, including human breast (MCF-7/WT, MCF7/ADR^R and MDA-231) and ovarian (OVCAR-3) carcinoma cells, Chinese hamster ovary (CHO) cells, rat hepatocytes (BRL 3A) and rat hepatoma (MH1C1) cells. Interestingly, and in contrast with the parent non-hydroxylated compound Tempo and its 4-amino-derivative Tempamine, Tempol was found to be significantly more cytotoxic against neoplastic than against non-neoplastic cell lines, with the following IC_{50} values (mM) in a 4-day MTT assay: 0.208 (MCF-7/WT), 0.222 (OVCAR-3), 0.410 (MCF-7/ADR^R), 0.571 (MDA- 231), 0.773 (MH1C1), 0.891 (CHO) and 1.073 (BRL 3A). Cellular pharmacokinetic data obtained in MCF-7/WT cells suggest that the hydroxylamine metabolite of Tempol is involved in its cytotoxic effects. Cell cycle studies indicate that cell death does not occur by apoptosis and that cell cycle effects are not prominent in the cytotoxicity of Tempol.

2305

O-aryl-N-(2-chloroethyl) carbamates as potential antineoplastic agents. C.-Gaudreault, R., Bomais, S., Lacroix, J., Poyet, P. and Mongrain C. Centre de recherche, Hôpital Saint-François d'Assise, Québec, Canada, G1L 3L5.

In the past few years we have developed a new class of antineoplastic agents: O-aryl-N-(2-chloroethyl) carbamates (CEC).



Structure-activity relationships indicated that CEC bearing $-\sigma, +\pi$ groups on the aromatic ring are highly cytotoxic against a wide variety of human and animal cancer cell lines, including colon adenocarcinoma (LoVo) and the hormone-independant breast cancer (MDA-MB-231) cell lines. The action mechanism of these molecules is not completely elucidated. However, preliminary experiments have shown that CEC are not alkylating agents. Furthermore, important modifications of the expression of tubulin by CEC were observed. This suggest that the antiproliferative activity of CEC is mediated by action mechanisms alike those of vincristine or podophyllotoxine.

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2306

Suppression of rhabdomyosarcoma growth by fumagillin analog TNP-470. Kalebic, Thea, Groeneveld, Geert-Jan, Helman, Lee J. Molecular Oncology Section, Pediatric Branch, National Cancer Institute, Bethesda, MD 20892.

Rhabdomyosarcoma (RMS) is the most frequent soft tissue sarcoma of childhood. Since advanced stage RMS continues to have a very poor prognosis, newer therapeutic approaches are needed. We investigated whether RMS growth may be suppressed by TNP-470, an analog of fumagillin, which was found to inhibit neoangiogenesis and to limit tumorigenic potential of different tumor cell lines. The antitumor effect of this compound was studied *in vivo* by treating tumor bearing mice twice weekly with different doses of TNP-470. The tumors were established in recipient animals by injecting subcutaneously a human rhabdomyosarcoma cell line RD. Treatment with TNP-470 suppressed, in a dose dependent fashion, the growth of RMS. When compared to controls, TNP-470-treated animals (60 mg/kg) had approximately 50% smaller tumors. We investigated also whether a decreased size of tumors in TNP-470-treated animals was a consequence of proliferative arrest, apoptosis or necrosis. This study suggested that agents inhibiting neoangiogenesis may find an application in new strategies for treatment of RMS.

2307

Reductive Metabolism of the Novel Pyrazoloacridone KW-2170 and Related Analogues *In vitro*. Holmes J[1], Patterson LH[2], Inoue K[3] and Graham MA[1].
[1]CRC Dept Medical Oncology, University of Glasgow, Scotland, UK, [2]Dept Pharmacy, DeMontfort University, Leicester, England UK, [3]Kyowa Hakko Kogyo Co. Japan.

The pyrazoloacridones are a new class of anti-tumour agent with broad spectrum activity and efficacy against multidrug resistant tumours. The reductive metabolism of KW-2170, doxorubicin and a number of pyrazoloacridone analogues was investigated to establish the ability of these compounds to catalyse free radical tissue damage. Redox cycling was investigated using NADPH fortified rat liver microsomes *in vitro*. Doxorubicin (25-200μM) stimulated NADPH oxidation, approximately two fold. In contrast, KW-2170 partially inhibited both basal rate and doxorubicin stimulated NADPH oxidation. KW-2170 had minimal effect on superoxide formation *in vitro* with levels barely above basal rate formation. Coincubation of KW-2170 inhibited doxorubicin stimulated free-radical formation, demonstrating that the pyrazoloacridones inhibited rather than stimulated redox cycling. ESR spectroscopy studies with KW-2170 failed to detect a drug free-radical signal under conditions readily producing a doxorubicin semiquinone free-radical. However, co-incubation of KW-2170 with an equimolar concentration of doxorubicin diminished the doxorubicin ESR signal by up to 59% over 30 minutes. Doxorubicin, stimulated lipid peroxidation by approximately three fold at 100μM. However, KW-2170 inhibited basal rate and doxorubicin induced lipid peroxidation by 62%. In summary, the pyrazoloacridones are not measurably reduced *in vitro* and actually inhibit endogenous and doxorubicin induced free radical generation. As a result they are likely to have an improved side effect profile compared to doxorubicin based on their inability to undergo redox cycling *in vitro*.

2308

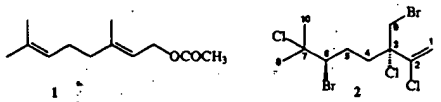
Evaluation of replicating DNA as a target for DNA damaging antitumor drugs. Cobuzzi, R.J., Jr., McHugh, M.M., Burhans, W.C., Beerman, T.A. Roswell Park Cancer Institute, Buffalo, NY 14263.

DNA replication is a controlling factor in cell division and provides an important chemotherapeutic target. In contrast to eukaryotic DNA replication, the mechanisms involved in viral replication are well defined. Our studies have focused on the examination of the effects of DNA-damaging antitumor agents on viral DNA relication in the Simian virus 40 (SV40) and Epstein Barr virus (EBV) systems. We used 2-dimensional (2-D) agarose gel electrophoresis to identify DNA damaging drugs capable of inhibiting such DNA replication processes as initiation, elongation, breakage and recombination of nascent DNA molecules within an intracellular environment. Two classes of drugs were studied: 1) Alkylating minor groove binding drugs with emphasis on the cyclopropylpyrroloindole (CPI) family of compounds, and 2) enediyne-containing strand scission agents. DNA lesions were quantitated by topological forms conversion and were correlated with effects on replication. Treatment of SV40-infected African green monkey kidney cells (BSC-1) with the CPI agents CC-1065 and adozelesin (U-73,975) produced unique gel patterns. CC-1065 induced the breakage of replication bubbles as evidenced by the appearance of fork arcs on 2-D gels with increasing drug concentration. Although adozelesin-treatment resulted in a disappearance of replication bubbles, there was no evidence of fork arc generation. Thus, while both CPI drugs are efficient inhibitors of DNA replication, the mechanisms by which they elicit their effects may differ. (Supported by NCI CA28495 and CA16056)

2309

Synthesis of, and Structure-Activity Relationship Studies with Halomon, a Novel Tumor Inhibitory Marine Toxin.

Anderson, W.K.; Bhamare, N.K.; Gavaskar, K.V.; Molnar, M.R.; Reddy, P.S.; Rubino, R.S. *Department of Medicinal Chemistry, School of Pharmacy, University at Buffalo, Buffalo NY 14260.*



Halomon (2) was synthesized in five steps from geranyl acetate (1). The nonstereospecific approach afforded four stereoisomers which were separated by chiral HPLC. Synthetic (+)-halomon was compared to an authentic sample (HPLC, CD, $[\alpha]_D$, ^{1}H- and ^{13}C NMR). The antitumor activities of three unnatural isomers of halomon were compared to halomon. The synthetic approach was used to prepare a range of halomon analogues with different halogenation patterns for antitumor evaluation. Structure activity relationship studies revealed that the halogen substitution pattern could be altered, but in a limited manner. Chemical studies with halomon showed it to be very unreactive toward nucleophiles. [Supported by the Drug Synthesis & Chemistry Branch, NCI (Contract NO1-CM-17569)].

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hours after the injection, the animals were sacrificed and the hearts were isolated for analysis of apoptosis. Apoptotic morphological alteration was identified by electron microscopy and was further confirmed by fluorescent microscopic examination of Hoechst 33258 staining. Results showed that doxorubicin induced apoptosis in the heart of nontransgenic mice and this drug effect was markedly suppressed in the MT overexpressing heart of transgenic mice. Supported in part by NIH Grant CA63752 and CA68125, and a Grant-in-Aid from the American Heart Association 947070.

#1294 Reversal of apoptosis resistance by butyrate in rat Nb2 lymphoma cells. Buckley, A.R., Buckley, D.J., Krumenacker, J.S., Leff, M.A., Magnuson, N.S., Reed, J.C., Miyashita, T., de Jong, G., and Gout, P.W. *University of North Dakota School of Medicine, Grand Forks, ND 58202, Washington State University, Pullman, WA 99164, Burnham Institute, La Jolla, CA 92037, B.C. Cancer Agency, Vancouver, B.C., Canada V5Z 4E6*

The highly malignant and autonomous Nb2-SFJCD1 cell line is resistant to dexamethasone (DEX)-activated cell death. Previously, we reported that exposure to butyrate (BT) transiently arrests growth in the G1 phase, reverts this line to a growth factor-dependent phenotype, and attenuates protooncogene expression (Cell Growth & Diff. 7:1713,1996). In the present study it was determined whether BT exposure alters sensitivity of Nb2-SFJCD1 cells to DEX-activated apoptosis. Pretreatment with BT (2 mM, 72h) reversed resistance to apoptosis in cells exposed to DEX (100 nM) for 12h as assessed by flow cytometry and DNA fragmentation on agarose gels. Mitogen (PRL, 20 ng/ml) stimulation abrogated the cytolytic effect of DEX. Evaluation of apoptosis-associated gene expression revealed that DEX significantly reduced *bcl-2* mRNA levels. The addition of PRL maintained *bcl-2* mRNA and significantly induced *pim-1* mRNA and protein expression. It is concluded that BT reverses resistance of Nb2-SFJCD1 cells to DEX-activated cell death. Moreover, the results suggest that mitogenic stimulation blocks critical components of apoptosis via a mechanism that reflects a requirement for *pim-1* and *bcl-2* expression.

#1295 p53 independent apoptotic cell death by cisplatin in SV-40 transformed cells with targeted disruption of metallothionein I and II genes. Kondo, Y., Yanagiya, T., Schwartz, D., Akimoto, M., Lazo, J.S., and Imura, N. *Nippon Medical School, Tokyo, Japan 113, Kitasato University, Tokyo, Japan 108, University of Pittsburgh, Pittsbugh, PA 15261*

Embryonic cells obtained from mice with targeted disruption of metallothionein (MT) I and II genes were immortalized with a plasmid encoding the SV-40 large T antigen. The resulting cell population expressed no detectable MT in contrast with immortalized wild type mouse embryonic cells (MT +/+). The MT null (MT-/-) cells were more sensitive to the growth inhibitory effects of cadmium, zinc, tert butyl hydroperoxide (tBH) and cisplatin. Compared with MT+/+ cells, MT-/- cells were more susceptible to apoptotic death after exposure to cisplatin. No significant differences were detected in the levels of glutathione, CuZn superoxide dismutase, glutathione peroxidase and catalase between the MT -/- and +/+ cells. MT null cells had also similar level of p53 and Bax protein, but elevated levels of c-myc mRNA. These results suggested that MT is p53 - independent apoptosis protector against cisplatin.

#1296 Activation of programmed cell death (apoptosis) by 4-tert-butyl-[3-(2-chloroethyl) ureido] benzene (tBCEU). Bourgoin, M., C-Gaudreault, R., and Poyet, P. *Department of Biochemistry, and Pharmacology, Faculty of Medicine, Laval University, and Centre Hospitalier Universitaire de Québec, Pavillon Saint-François d'Assise, Québec, Canada, G1L 3L5*

4-tert-butyl-[3-(2-chloroethyl) ureido] benzene (tBCEU) is a member of a novel family of antineoplastic agents; 1-aryl-3-(2-chloroethyl) ureas. tBCEU has shown potent biological activity both *In vivo* and *In vitro*. This molecule seems cytotoxic through microtubule depolymerization. tBCEU is also active on cell lines that are resistance to anticancer agents through the overexpression of the P-glycoprotein, the alteration in GSH metabolism and/or GST activity, the alteration of topoisomerases and the increase in DNA repair capacity. Since the cellular resistance to anticancer agents is often related to the capacity of cells to undergo apoptosis and that eukariotic cells can die by apoptosis when treated with cytotoxic drugs, we have evaluated the cellular mechanism of death when cells are treated with tBCEU. The assay for the apoptosis consisted at first, to incubate Chinese hamster ovary cells (CHO-10001) during 1 hour with either tBCEU (1, 3, 10, 30, 100 μM) or colchicine (10 μg/ml). Afterward, the cells were washed and cultured in a regular media during 48 hours prior to the assays. The presence of apoptosis was evaluated by DNA gel electrophoresis, fluorescence microscopy, and flow cytometry analysis. We have found that tBCEU induces apoptosis in a dose dependent manner. Furthermore, this induction of apoptosis was blocked by the addition of the protein synthesis inhibitor cycloheximide. Altogether, the data indicated that tBCEU might be a potent apoptosis inductor. (This work is supported by a grant from the National Cancer institute of Canada).

#1297 Induction of apoptosis by sodium selenite and selenodiglutathione in HL-60 cells. Cho, D.Y., and Chung, A.S. *Korea Advanced Institute of Science and Technology, Taejon, Korea 305-701*

Apoptosis induced by selenium was examined in human promyelocytic leukemia (HL-60) cells. Cytoplasmic histone-associated DNA fragments were increased by the treatment of selenodiglutathione (SDG) and selenite in dose dependent manner. The DNA fragmentation was detected by sandwich-enzyme immunoassay using mouse monoclonal antibodies directed against DNA and histones. These results were also confirmed by agarose gel electrophoresis of DNA from HL-60 cells. The profiles of apoptosis induced by selenite and SDG were compared in an attempt to assess the correlation between induction of apoptosis by selenium and the reductive metabolism of selenite. A time-course experiment showed that DNA fragmentation induced by SDG and selenite was observed after 10 and 12 hours, respectively. However, time-course profiles of cellular selenium uptake and glutathione (GSH) depletion of SDG-treated cells were shown to be faster more than 10 hours compared with those of selenite-treated cells. It was, moreover, observed that pre-depletion of GSH (<10% of control) by treatment of buthionine sulfoximine didn't exert an effect upon the apoptosis profile induced by selenite or SDG. Therefore, these results suggested that crucial intermediate of selenium inducing apoptosis in HL-60 cells may not belong to the general reductive pathway of selenite by GSH.

#1298 DNA damage and apoptotsis in human leukemic cells treated with the piperidine nitroxide TEMPOL. Monti, E., Gariboldi, M.B., Supino, R., and Piccinini, F. *Inst. of Pharmacology, Univ. of Milan, Italy, Istituto Nazionale Tumori, Milan, Italy*

Piperidine nitroxide radicals act as antioxidants in several free radical-mediated pathologies (Krishna and Samuni, Meth Enzymol. 234: 580, 1994). A recent study showed that these compounds are mutagenic as well as cytotoxic against DNA repair-deficient bacterial strains (Wang *et al*, Biochim Biophys Acta 1305: 71, 1996). This observation suggested that similar effects might be elicited in tumor cell lines. In the present study we evaluated the cytotoxicity of 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPOL) against two human leukemic cell lines, HL-60 and KG1. Our results show that HL-60 cells are more sensitive than KG1 (IC_{50} 0.35±0.08 mM and 1.3±0.14 mM, respectively for 96-h exposure, M±SE). Analysis of DNA fragmentation by agarose gel electrophoresis and filter binding assay in TEMPOL-treated HL-60 cells showed a dose-dependent effect, which was absent in KG1 cells. The two cell lines exhibited different cell cycle distributions following TEMPOL treatment, with a partial G_1 block for KG1 and a shift towards S and G_2/M phases for HL-60. Cell cycle studies also evidenced a dose- and time-dependent increase of apoptotis for HL-60 but not for KG1 cells. Immunoblot analysis of bcl-2 indicated the presence of higher protein levels in KG1 than in HL-60 cells. We conclude that cytotoxic effect of TEMPOL in human leukemic cells is related to induction of apoptosis. CNR grant no. 96.03345.CT04.

#1299 Antimitotic drugs induce cyclin dependent kinase activity and hyperphosphorylation of the retinoblastoma protein. Emanuel, S., and Cohen, D. *Oncology Research Group, Preclinical Research, Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, East Hanover, NJ 07936*

The antimitotic drugs taxol and vinblastine were examined for their non-microtubule related effects on MDA-435 human breast carcinoma cells. Both drugs increased cdk2 and cdk4 activity and induced hyperphosphorylation of RB. In addition, the two drugs stimulated cdc2 activity and the MAPK signal transduction pathway. The activity of the MAPK pathway elements and cyclin dependent kinases were found to be elevated in two multidrug resistant (MDR) cell lines that were selected with taxol and express high levels of P-glycoprotein and hyperphosphorylated RB. Furthermore, expression of the RB-regulated proteins cdc2, cyclin A and myc was also found to be higher in MDR cells. This was manifested in a higher growth rate for MDR cells in low serum. When MDR cells were grown without taxol, the high levels of protein expression and kinase activity persisted for up to 104 days before decreasing. These observations suggest that despite high levels of P-glycoprotein in MDR cells, a sufficient amount of taxol remains intracellular to accelerate the cell cycle machinery and activate the MAPK signal transduction pathway leading to a more transformed phenotype. Thus, in addition to the development of MDR, exposure of tumor cells to cytotoxic agents produces further changes that result in enhancement of chemotherapy resistance.

#1300 Expression of Fas ligand by human lung cancers. Frizzelle, S.P., Brunner, T., Niehans, G.A., Liston, J.C., Salerno, C.T., Knapp, D.J., Green, D.R., and Kratzke, R.A. *Dept. of Pathology [G.A.N., D.J.K.], Dept.of Medicine, Section of Hematology/Oncology [S.P.F., J.C.L., R.A.K.], and the Dept. of Surgery [C.T.S.], Minneapolis Veterans Affairs Medical Center and the University of Minnesota Medical School, MN 55417, La Jolla Institute of Allergy and Immunology, San Diego, CA 92121 [T.B., D.R.G.]*

In order to reach a clinically detectable size neoplasms must be able to suppress or evade a host immune response. Activated T cells may enter apoptosis in the presence of Fas ligand (FasL), and tissue expression of FasL has been shown to contribute to immune privilege in the eye and testis. We have demonstrated that all human lung carcinoma cell lines tested (16 of 16) express a 38-kilodalton protein consistent with FasL by immunoblotting, while the majority of resected tumors (23 of 28) show positive staining for FasL by immunohistochemistry. DNA sequencing of RT-PCR products from lung cancer cells and resected lung tumors confirms the presence of human FasL mRNA in these neoplastic tissues. Furthermore, lung carcinoma cells are capable of killing a Fas-sensitive human T cell line (Jurkat) in co-culture experiments; this killing was inhibited by a recombinant form of the soluble portion of the Fas receptor (FasFc).

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H322 cell line showed 100% positive staining. P21 overexpression was seen in G0/G1 but also in G2/M phase arrested cells indicating a dual role of this protein in regulating G0/G1 but also G2/M transition. [Supported by an ECC fellowship]

#609 5-Fluorouracil causes p53-dependent cytotoxicity to HCT116 cells, yet is acting predominantly by RNA damage rather than by thymidylate synthase inhibition. Pritchard D.M. and Hickman J.A. *G38 Stopford Building, School of Biological Sciences, University of Manchester, UK, M13 9PT.*

We have previously demonstrated that 5-Fluorouracil (5FU) induces p53-dependent apoptosis in the murine intestine *in vivo*, yet this apoptosis results predominantly from RNA directed cytotoxicity (PNAS (1997) **94**:1795–9). An *in vitro* model of this has now been established using HCT116 human colon carcinoma cells: 5FU treatment induces p53 and $p21^{WAF1/CIP1}$ expression at times (6 hr) when no strand breaks can be observed in nascent DNA by alkaline elution. The cytotoxic effects of 10μM 5FU to HCT116 cells are reduced by administration of 1mM uridine, but are not affected by 25μM thymidine (though 25μM thymidine almost completely abolishes the cytotoxic effects of 100nM Tomudex (ZD1694), a pure thymidylate synthase inhibitor). Less p53 induction is observed in cells cotreated with 2.5μM 5FU and 1mM uridine compared to cells treated with 2.5μM 5FU alone. Stable clones have been isolated following transfection of HCT116 cells with human papilloma virus E6. These show greatly reduced levels of p53 protein, both in the untreated state and following treatment with 5FU or Etoposide. E6 transfected cells are significantly more resistant to 10μM 5FU. 5FU appears to act more by thymidylate synthase inhibition in E6 transfected cells, as the cytotoxicity of 10μM 5FU can be reduced by coadministration of 25μM thymidine. In HCT116 cells therefore, 5-FU induced cytotoxicity results largely from RNA damage, rather than DNA damage following thymidylate synthase inhibition. This cytotoxicity is partly p53 dependent, which suggests a novel role for p53 in the recognition of RNA damage itself or its consequences.

#610 The piperidine nitroxide TEMPOL induces apoptosis and $p21^{waf1/cip1}$ expression in p53-deficient cells. Monti, E., Gariboldi, M.B., Grossi, S., Lucchi, S., Supino, R. *Istitute of Pharmacology, University of Milan, I20129 Milan, Italy, and Istituto Nazionale Tumori, I20133 Milan, Italy.*

The piperidine nitroxide TEMPOL has been shown to inhibit the growth of several mammalian cancer cell lines. In human adenocarcinoma MCF-7 cells expressing functional wildtype p53, this occurs through the induction of apoptosis. As the cytotoxicity of TEMPOL seems to depend on its free radical nitroxyl moiety, cell damage has been proposed to occur by free radical attack to the DNA involving p53-dependent transcription activation of proapoptotic genes (Gariboldi et al., *Free Rad Biol Med*, 1997, in press). The aim of the present study was to verify that TEMPOL can induce apoptotic cell death in HL-60 cells, which are known to lack p53, and to identify the gene products involved in its cytotoxic effect. Flow cytometry, fluorescence microscopy and DNA fragmentation studies of TEMPOL-treated HL-60 cells are consistent with an apoptotic mechanism of cell death. In spite of the absence of p53, TEMPOL induces a time-dependent $p21^{waf1/cip1}$ expression, which precedes the onset of the effector phase of apoptosis. This effect is abrogated by cotreatment with N-acetylcysteine, indicating an underlying free radical-mediated mechanism. Members of the Bcl-2 family of proteins (Bax and Bcl-2) are unmodified by TEMPOL treatment. Therefore our findings strongly support a role for $p21^{waf1/cip1}$ in TEMPOL-induced apoptosis in p53-dependent cells.

#611 The role of Bcl-2 and NFκB in the induction of cell death by anthracyc-lines. Polka, D.C., Schaefer, A. and Marquardt, H. *Fraunhofer Dept. of Toxicology, Grindelallee 117, 20146 Hamburg, Germany.*

The anthracycline antitumor antibiotics are widely used in cancer chemotherapy. However, little is known about the exact biochemical mechanisms of their cytostatic action. We have therefore investigated the capability of three derivatives -Aclarubicine (ACL), Doxorubicine (DOX) and Daunorubicine (DAU)- to induce cell death in the human leukemic cell line HL-60. After treatment with ACL, 50nM, the cells exhibited an apoptotic morphology within 24h, whereas equitoxic concentrations of DOX and DAU (20nM) induced predominantly morphological changes resembling necrosis. In addition to that, ACL, 75nM, induced oligosomal DNA fragmentation after 3h, in contrast to DOX and DAU, where no fragmentation occurred, even after treatment with 200 and 300nM, respectively. Also, ACL reduced the level of the antiapoptotic protein Bcl-2, while DOX and DAU had no effect on this protein. The level of its counterpart Bax was not influenced by the anthra-cyclines studied. The role of the transcription factor NFκB has currently been considered in the regulation of apoptosis. Therefore, we have examined the effect of the anthracyclines on the NFκB DNA-binding activity in HL-60 cells. Activation could be detected with all substances at a concentration of 500nM. However, at lower concentrations, at which ACL induced DNA-degradation, morphological changes characteristic for apoptosis and Bcl-2 down-regulation, no activation of NFκB was observed. These results suggest that the structure-specific induction of apoptosis by ACL is most probably due to the down-regulation of Bcl-2 levels and is not related to an activation of NFκB.

#612 Role of Bcl-2 Phosphorylation in Mitotic Arrest and Apoptosis in HeLa Cells Treated with Antitubulin Agents. Ling Y.H., Tornos, C., and Perez-Soler, R. *The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.*

We studied the correlation between bcl-2 phosphorylation, mitotic arrest, and apoptosis caused by taxol and nocodazole in Hela cells. Continuous exposure of HeLa cells to 50 ng/ml taxol resulted in M-phase arrest, cyclin B1 accumulation, and cdc2/cyclin B1 kinase activation, all peaking at 24 h and then gradually declining to baseline at 48–60 h. In contrast, apoptosis was first detected at 12 h and steadily increased thereafter, 70–90% cells being apoptotic at 48–60 h. Bcl-2 phosphorylation determined by PAGE was closely associated with M-phase arrest but not with apoptosis. Similar results were obtained in cells synchronized with nocodazole and double thymidine. The phosphatase inhibitor okadaic acid inhibited dephosphorylation of bcl-2 and delayed the progression of M-phase cells into interphase. The serine/threonine kinase inhibitor staurosporine accelerated the dephosphorylation of phosphorylated bcl-2 and the progression of M-phase arrested cells into interphase. Immune complex kinase assays in cell free systems showed that the bcl-2 protein can be a substrate of cdc2/cyclin B1 kinase isolated from taxol-arrested M-phase cells. Our data suggest that phosphorylated bcl-2 is a marker of mitosis and not a determinant of progression from mitosis to apoptosis. Supported by CA50270.

#613 A functional role for protein kinase Cα in Bcl2 phosphorylation and suppression of apoptosis. Ruvolo, P.P., Carr, B.K. and May, W.S. *University of Texas Medical Branch at Galveston, Galveston, Texas 77555-1048.*

Phosphorylation of Bcl2 at serine 70 has been shown to be required for suppression of apoptosis in murine growth factor-dependent cell lines (JBC 272: 1161–1163, 1997). Human pre-B REH cells express high levels of Bcl-2 yet remain sensitive to the active chemotherapy drugs etopside, araC, and adriamycin, while the myeloid leukemia derived HL60 cells express ~ one half the level of Bcl-2 but are >10 fold more resistant to these drugs. The mechanism for this apparent dichotomy appears to involve a deficiency in REH cells of PKCα isoform localization to mitochondrial membranes since (1) HL60 cells, which are resistant to chemotherapy, contain highly phosphorylated Bcl-2, while REH cells demonstrated little or no basal phosphorylation; (2) PKCα was the only classical isoform found to co-localize with Bcl2 in mitochondrial membranes from HL60 cells but was absent in parental REH cells; (3) the potent PKC activator, bryostatin-1, could induce Bcl-2 phosphorylation in REH cells in association with PKCα co-localization with Bcl-2; (4) bryostatin-1 treatment could increase the resistance of REH cells to drug-induced apoptosis; and (5) stable forced expression of the PKCα isoform in REH cells induced mitochondrial PKCα localization in close association with increased phosphorylation of Bcl2 and >10 fold increase in resistance to chemotherapy-induced apoptosis versus empty vector or parental cells. Since bryostatin-1 treatment of the PKCα transformants leads to an even higher level of Bcl2 phosphorylation and drug resistance, these findings indicate a functional role for PKCα and suggest potentially novel therapeutic strategies.

#614 Arsenic trioxide induces apoptosis of myeloid leukemia cells by activation of caspases via an alternative transduction pathway. Xiao-jun Huang, Peter H. Wiernik, Robert S. Klein, Robert E. Gallagher. *Department of Oncology, Montefiore Medical Center, Bronx, NY 10467.*

Arsenic trioxide (ATO) was recently reported to induce apoptosis in the myeloid leukemia cell lines NB_4 and HL-60 cells. We tested these cells for the involvement of caspase protease, which have been identified as the final mediators of apoptosis for many bioactive agents, in response to ATO. We found that one common substrates for several caspases, poly (ADP[adenosine 5'-diphosphate]-ribose) polymerase (PARP), was cleaved in both NB_4 and HL-60 cells within 12 hours exposure to ATO (1 μM). Further, Incubation of either cell type with the broad-spectrum caspase inhibitor Z-VAD.fmk or with a high concentration of the caspase 1 inhibitor Y-VAD.cho (600 μM) completely prevented ATO-induced apoptosis of NB_4 and HL-60 cells. Pre-treatment of these cells with the protein kinase C activator 12-o-tetradecanoylphorbol 13-acetate (TPA), under conditions that have been reported to inhibit apoptosis by the chemotherapeutic agent VP-16, did not prevent ATO-induced apoptosis of HL-60 or NB_4 cells. These results indicate that caspase-activation plays an important role in ATO-induced apoptosis of myeloid leukemia cells but suggest that ATO and VP-16 produced apoptosis by alternative transduction pathways.

#615 Relationships between caspase-3 activation, G2/M arrest and 50 kilobase (kb) DNA breakage during etoposide-induced death of human leukemic cells. Stewart, Bernard W. and Sleiman, Robert J. *Children's Cancer Research Institute, Children's Cancer Institute Australia, Sydney Children's Hospital, Sydney, NSW 2031, Australia.*

Human leukemic lines exposed to 5μM (or more) etoposide die within 6 h. Using low etoposide concentrations (0.5μM or less), death of CEM or MOLT-4 lymphoblastoid cells occurs 48 h after addition of the drug, and is preceded by G2/M cell cycle arrest. Under these conditions, activation of caspase-3 (CPP32/Yama/apopain) is evident from increased mRNA (RT-PCR) and protein (Western) levels, together with cleavage of poly (ADP-ribose) polymerase and increased enzymic activity in correponding cell fractions. Caspase-3 activation occurs within 3h of drug treatment, and is not a consequence of G2/M arrest, the latter being most marked 24h drug treatment. G2/M arrest is correlated with increased